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Dredged Material Evaluations: Review of Zooplankton Toxicity Test Methods for Marine Water Quality Evaluations

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PURPOSE: The first objective of this Dredging Operations and Engineering (DOER) technical note is to summarize currently available estuarine and marine water-only zooplankton toxicity test methods that may be suitable for use (with or without modifications) in dredging evaluations that are being conducted under the Marine Protection Research and Sanctuaries Act (103 evaluations). The second objective is to identify additional zooplankton species that are available commercially but do not have established test methods. Development of toxicity test methods should be considered for these marine zooplankton species.

BACKGROUND: Dredging of ports and harbors is necessary to maintain navigable waterways. However, large volumes of dredged sediment or dredged material (DM) must be managed. Federal regulations require the physical, chemical, and toxicological evaluation of DM to determine if it is suitable for unrestricted open water placement, beneficial use, or if management strategies are necessary. Open water placement of DM into inland water bodies or coastal areas inland of the national baseline is regulated under the Clean Water Act (CWA). Open water placement into the ocean is regulated under the Marine Protection Research and Sanctuaries Act (MPRSA). Toxicological evaluations of DM involve testing the potential for short-term impacts of the DM while settling through the water column (elutriate toxicity tests), and long-term toxicity (whole sediment toxicity tests) and accumulation of contaminants of concern into biological tissue (bioaccumulation tests) after sediment settles at the placement site. Technical guidance for performing DM evaluations under MPRSA and CWA is provided in the Ocean Testing Manual (USEPA/USACE 1991) and Inland Testing Manual (USEPA/USACE 1998), respectively. While these documents provide valuable information, including toxicological test methods, they require updating.

Sediment elutriates are prepared according to guidance (USEPA/USACE 1991; 1998) by mixing sediment and site water and allowing settling for prescribed periods. Additional detail on the creation of sediment elutriates for toxicity testing can be found in Kennedy et al. (2015). Generally, recommended species for elutriate toxicity tests are fish, invertebrates (crustaceans), and zooplankton. Freshwater evaluations (following CWA regulations) use standard test organisms and methods covering fish and zooplankton taxa (USEPA 2002). The MPRSA requires greater specificity on the type and number of test species used in testing. According to 40 CFR §227.27 (Limiting Permissible Concentration 2012), "*appropriate sensitive marine organisms means at least one species each representative of phytoplankton or zooplankton, crustacean or mollusk, and fish species chosen from*

among the most sensitive species documented in the scientific literature or accepted by EPA as being reliable test organisms to determine the anticipated impact of the wastes on the ecosystem at the disposal site". This effectively requires three elutriate toxicity test methods to be employed for each evaluation conducted under MPRSA. For additional information on how these biological tests relate to different compartments of the placement site, with a heavy focus on the conduct of water column toxicity evaluations of DM, see Kennedy et al. (2015).

TOXICITY TESTS USING PLANKTONIC EMBRYOS: The American Society for Testing and Materials International (ASTM) has produced standardized toxicity tests for echinoderm (ASTM E1563-98[2004]) and bivalve (mussels, oysters) embryo development (ASTM E724-98[2004]); these test methods are currently being used as zooplankton tests for MPRSA evaluations in some regions. The organisms used in these test methods are not planktonic for most of their life cycles (juveniles and adults are benthic). Only their short-duration larval stages are planktonic. Therefore, they are not holozooplankton (defined as animals that are planktonic for their entire life cycle). Further, these embryo and larval life stages are particularly sensitive to non-persistent contaminants (e.g., ammonia) that are commonly present in elutriate water, and may cause toxicity, thus confounding the toxicity assessment of persistent contaminants of concern (e.g., metals and hydrophobic organic compounds). For an extensive discussion of these development toxicity tests in the context and practice of DM evaluations including issues in applying application factors for survival (LC50) data to development toxicity data (EC50), see Kennedy et al. (2015).

TOXCITY TESTING USING HOLOZOOPLANKTON: Testing using marine animals that are planktonic for all of their life cycle is needed to satisfy the MPRSA zooplankton requirement. Test species should be relatively sensitive to persistent contaminants of concern but less sensitive to confounding factors, such as handling, physical particle effects and non-persistent contaminants. Table 1a lists candidate marine holozooplankton test species that are commercially available in the United States, and have toxicity test methods at various stages of development (literature published or standardized methods). This species list is not intended to be comprehensive; instead it serves to illustrate that holozooplankton species are available for use in routine testing of DM elutriates. It also prioritizes marine holozooplankton species that are readily commercially available year round, a characteristic that is key to biological testing of DM (USEPA/USACE 1991; 1998).

Table 1. List of candidate marine holozooplankton test species (a) and epibenthic species (b). While holozooplankton species are generally more relevant for elutriate or water column evaluations, harpacticoid copepods (b) are listed separately since more testing has been conducted in the United States and more test methods are available.

(a)

Taxon	***Genus***	***Species***
Calanoid copepods	*Acartia*	*A. tonsa, A. sinjiensis*
	Pseudodiaptomus	*P. pelagicus, P. salina*
	Parvocalanus	*P. crassirostris*
Harpacticoid copepod	*Euterpina**	*E. acutifrons*
Cladoceran	*Moina*	*M. salina, M. monogolica*
Rotifer	*Brachionus*	*B. plicatilis*

* While harpacticoids are typically benthic, *Euterpina* is an exception.

(b)

Taxon	***Genus***	***Species***
Harpacticoid copepods	*Amphiascus* *Nitocra*	*A. tenuiremis* *N. spinipes*
	Tisbe	*T. biminensis*

STANDARDIZED TESTS FOR HOLOZOOPLANKTON: A standardized 24-hour acute toxicity test method is currently available for freshwater rotifers, which includes an appendix to modify those methods for the estuarine/marine rotifer *Brachionus plicatilis* (ASTM E1440-91 [2012]). The International Organization for Standardization (ISO) has published a marine test method for *B. plicatilis* (ISO/FDIS 19820 [2016]). Studies from Europe and Asia have led to efforts to standardize copepod toxicity tests by various organizations, such as the ISO, Organisation for Economic Cooperation and Development (OECD) and ASTM. Much of this work has focused on full life-cycle testing of the epibenthic harpacticoid copepods, rather than the holoplanktonic calanoid copepods; and therefore, has lower relevance to testing toxicity in the water column. For example, a published method exists for a copepod life cycle test using the marine meibenthic copepod *Amphiascus tenuiremis* (ASTM E2317-04 [2012]) and *Nitocra spinipes* (ISO/DTS 18220[1]). Some guidance document validation work (in draft form) did involve the calanoid *Acartia tonsa* (OECD 2007; ASTM STP667 [1979]) and a full life cycle method (ISO 16778 [2015]) has been published. For acute toxicity, calanoid copepods (*Acartia* spp.) are listed in ASTM E1850-04 (2012); however, the testing methods are generalized to a large number of freshwater, saltwater, invertebrate and vertebrate test organisms and thus do not provide adequate species specific detail. An acute lethality method has been published for the marine copepods *A. tonsa, Tisbe battagliai and N. spinipes* (ISO/DIS14669 [1999]), but only *A. tonsa* is holozooplanktonic. No known standard methods are available for marine

[1] International Organization for Standardization (ISO). In review. Water quality - larval development test with the harpacticoid copepod *Nitocra spinipes* ISO/DTS 18220. International Organization for Standardization: Geneva, Switzerland.

cladocerans (*Moina* species). Work remains to be done, especially for establishing and standardizing a test method specific to water column elutriate toxicity testing for dredging evaluations conducted under MPRSA. General testing methods from available effluent discharges (USEPA 2002) and dredging evaluations (USEPA/USACE 1991; 1998) may be leveraged. Available feeding and culturing methods may be adapted to ensure food is not a limiting factor in cultures. However, it is appropriate to investigate lower feeding rations, or absence of food, in toxicity testing to reduce interactive effects between food and contaminants of concern.

PUBLISHED RESEARCH USING ALTERNATIVE ZOOPLANKTON: While a few standard test methods for marine holozooplankton are currently available (above section), greater culturing and acute toxicity testing methods are available in the published literature for certain species of marine copepods, cladocerans and rotifers. Most studies found in this review on the use of marine holozooplankton (e.g., copepods, cladocerans, rotifers) in toxicity testing were conducted outside the United States. However, many conspecifics reside in the coastal waters of the United States and therefore testing of these organisms as representative zooplankton species has high relevance. Researchers have previously described some marine copepod culturing methods scaled down from aquaculture for ecotoxicology testing (APHA 1989; Hall et al. 1997; Medina and Barata 2004; Gorbe et al. 2012; ISO/DIS14669 (1999). Various culturing densities were recommended, ranging from 250 to 1000 individuals per liter (APHA 1999; Medina and Barata 2004). Copepods reproduce sexually, with an assumed sex ratio of approximately 1:1 (Kusk and Petersen 1997). Feeding rations described in the literature range from simple 1:1 algae mixtures, such as *Isochrysis galbana* and *Rhinomonas reticulate* (Medina et al. 2002; Medina and Barata 2004), to more complex algae mixtures of *Skeletonema costatum*, *Thalassiosira pseudonana*, *I. galbana*, *R. baltica* (APHA 1999) or *I. galbana*, *Tetraselmis suecica*, and *R. reticulate* (Gorbe et al. 2012). While most copepod aquaculture and testing has been done in natural seawater, Kusk and Wollenberger (1999) provided data suggesting culture and testing of *A. tonsa* in artificial seawater is viable. Acute toxicity testing methods (48 to 96 hours) have been conducted and described in the literature for the marine calanoid copepods *A. tonsa* (Kusk and Petersen 1997; Kusk and Wollenberger 1999; Sverdrup et al. 2002; Medina et al. 2002; Gorbe et al. 2012; Bielmyer et al. 2006), *A. sinjiensis* (Rose et al. 2006; Gissi et al. 2013), *Pseudodiaptomus coronatus* (Hauch et al. 1980), *Pseudodiaptomus marinus* (Huang et al. 2006) and *E. affinis* (Hall et al. 1997). However, these test descriptions do not provide all of the details necessary for adapting the tests for use with DM elutriates. Feeding may be required during acute toxicity testing (e.g., Hauch et al. 1980), but this may result in interactions with contaminants of concern. Considerably more work has been conducted on the epibenthic harpacticoid copepods (e.g., Verriopoulos and Dimas 1988; Bechmann 1994; see also associated OECD 2007, ASTM and ISO standard methods).

Non-standardized culturing and acute toxicity testing methods for the marine cladoceran *Moina monogolica* have been described in the literature (He et al. 2001; Wang et al. 2007a; Wang et al. 2007b; Wang et al. 2009; Wang et al. 2010). However, selection of the test species and establishment of successful culture methods has been more elusive compared with to work with copepods (van Dam et al. 2008). These cladocerans reproduce

parthenogenetically, consume commercially available algae (e.g., *Chlorella* spp.), have a wide salinity tolerance (He et al. 2001) and can be cultured in synthetic seawater (Wang et al. 2010). Feeding rations have ranged from 2.5 x 10^3 to 3 x 10^6 cells/mL, while keeping adult cladoceran density below 50 individuals per liter (Wang et al. 2007a; Wang et al. 2007b; Wang et al. 2010). Substantially less culturing and toxicity testing information is available for *Moina* species relative to copepods, although *Moina* spp. offer advantages of asexual reproduction and can be tested for 48–hours in absence of food.

Culturing and testing methods (48–hour) have also been published for rotifers, particularly *Brachionus plicatilis* (Theilacker and McMaster 1971; Arnold et al. 2010a). This species is widely distributed throughout the world, very sensitive to metals such as copper, amenable for culture in artificial seawater (wide salinity range) and does not require feeding (for at least 80 hours) during acute testing (Arnold et al. 2010a). These authors increased the exposure duration from the 24-hour duration recommended in the ASTM method to enhance the sensitivity of endpoint response (e.g., decrease in copper LC50 from to 68 to 10 µg/L).

SENSITIVITY TO METALS: It is desirable that zooplankton test species be highly sensitive to persistent contaminants of concern. Marine copepods have a demonstrated relatively high sensitivity to metals, organometals, organic compounds and surfactants compared to other species (van Dam et al. 2008). Generally, calanoid copepods appear more sensitive to metals relative to harpacticoid copepods (Wang et al. 2007b). Rose et al. (2006) reported that a subtropical species of *Acartia* was one of the most sensitive species to copper in a species sensitivity distribution. *Acartia* was also more sensitive than bacteria, microalgae, urchins, oysters, prawn and fish (Kusk and Petersen 1997; Gissi et al. 2013). *Moina* is reported to be highly sensitive to metals (Garcia-Garcia et al. 2006; Wang et al. 2009). There is some evidence that *M. monogolica* is more sensitive to copper than harpacticoid copepods (i.e., *Tigriopus* spp.) and calanoid copepods (*Pseudodiatomus coronatus*, *Tisbe holothuriae*) but more tolerant than other calanoids (*Eurtemora affinis, Acartia* spp.) (Wang et al. 2007b). Arnold et al. (2010b) reported that an acute *E. affinis* test was not as sensitive to copper as echinoderm and mussel embryo development tests. The rotifer *B. plicatilis* was reported to be one of the most sensitive marine organisms to copper (Arnold et al. 2010a). A non-comprehensive summary of copper toxicity reference values for zooplankton species is provided in Table 2.

CONCLUSIONS AND PATH FORWARD: While acute echinoderm and bivalve embryo development toxicity tests are currently used to meet the zooplankton testing requirement for elutriate toxicity tests in some regions conducted under MPRSA dredging evaluations, a diversity of other true holozooplankton species have been identified herein (Table 1) that meet MPRSA zooplankton requirements. Standardized test methods (ASTM, ISO) exist for harpacticoid copepods; however, those copepod species are epibenthic and may be less appropriate for water column evaluations of DM. Standardized tests are also available for holozooplanktonic calanoid copepods (ISO) and a modification of a freshwater rotifer method is available (ASTM) for use with marine species. Marine cladocerans of the genus *Moina* are also good candidates for further method development. Further work is needed to validate these methods for the elutriate toxicity tests that are used in water column

evaluations of DM. Towards that end, future research at ERDC will select candidate holozooplankton species (Table 1a), validate proposed culturing, feeding, handling, and test conditions (Table 3) and determine relative sensitivity to metals and ammonia. The impacts of temperature and presence of food on toxicity will be considered. Ultimately, the testing development data and methods will be published in the peer reviewed literature and validated as test methods specific to elutriate toxicity tests for inclusion in future dredging evaluation guidance manuals.

Table 2. Sensitivity of select marine zooplankton to copper and ammonia.

Common name	Genus	Chemical	Endpoint	Concentration (µg/l)	Reference
Copepod (calanoids)	*Acartia*	*Copper*	*48h EC50*	*33*	Gissi et al. 2013
		Copper	*48h EC50*	*104-311*	Reeves et al. (1976) from Arnott and Ahsanullah(1979)
		Copper	*48h EC50*	*58*	Kwok et al. (2008)
		Copper	*48h EC50*	*200*	Arnott and Ahsanullah (1979)
		Copper	*48h EC50*	*21 (16 – 26)*	Rose et al. (2006)
		Ammonia	*48h NOEC*	*1,000 (50 unionized)**	Rose et al. (2006)
		Ammonia	*48h EC50*	*10,000 (500 unionized)**	Rose et al. (2006); Gissi et al. (2013)
	Eurytemra	*Copper*	*48h LC50*	*83*	Hall et al. (1997)
	Paracalanus	*Copper*	*24h LC50*	*190*	Arnott and Ahsanullah (1979)
Cladoceran	*Moina*	*Copper*	*48h LC50*	*106 (100 – 112)*	Wang et al. (2007b)
		Ammonia (unionized)	*NOEC*	*<2,600*	He et al. (2001)
		Ammonia (unionized)	*48h LC50*	*7,520*	He et al. (2001)
Rotifer	*Brachionus*	*Copper*	*48h LC50*	*10.1 (5.3 – 12.5)*	Arnold et al. (2010a)

unionized ammonia calculated with the following assumptions (total ammonia was total ammonia-N, temperature = 27 °C, salinity = 27‰, pH = 7.8).

Table 3. Summary of proposed test conditions for elutriate toxicity tests, adapted from the literature. Note that these conditions need to be tested and validated.

Parameter	***Copepod*** **(e.g., *Acartia* spp.)**	***Cladoceran*** **(*e.g., Moina* spp.)**	***Rotifer*** ***(e.g., Brachionus* spp.)**
Standard method	ISO/DIS14669 (1999) And adapted from cited literature	No	ASTM E 1440-91, modified for saltwater (Arnold et al. 2010a)
Temperature (°C)	20 (± 2) 25 – 27 in literature studies	20 (± 1)	25 (± 1)
pH	7-9	7-9	Adjust to 8
Salinity (‰)	25 - 30	10 – 30	15 (ASTM E 1440-91) 6 – 29 (Arnold et al. 2010a)

Parameter	*Copepod* (e.g., *Acartia* spp.)	*Cladoceran* (*e.g., Moina* spp.)	*Rotifer* (*e.g., Brachionus* spp.)
Duration (hours)	48	48	24 48 (Arnold et al. 2010a)
Age	Adults / sub-adults	<24 hours	0 – 2 hours 0 – 4 hours (Arnold et al. 2010a)
No organisms/ rep	5	5 to 10	10
No. reps (modified for elutriate testing)	5	5	5
Total organisms/concentration	25 (minimum)	25 to 50	50
Feeding	2 hours prior to test. No feeding during test (preferred) or at 0 hours if required for survival	2 hours prior to test; none during test	Prior to test; none during test
Aeration	Only if needed to maintain acceptable dissolved oxygen	Only if needed to maintain acceptable dissolved oxygen	Only if needed to maintain acceptable dissolved oxygen
Assessment endpoint	Immobilization	Survival, immobilization	Mortality (lack of movement for 5 seconds)
Test acceptability	≥90% in control	≥80-90% in control	≥90% in control

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This DOER-TN and files for the examples may be downloaded from *http://chl.wes.army.mil/library/publications/chetn/* and *http://xmswiki.com/*.

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